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# Evaluation of Feed Supplement as Antioxidant Source to Rumen Ecology

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**Abstract:** A study on utilization of feed supplement (FS) contains pineapples rind antioxidant (PrAOx) and mineral plus vitamin (MV) as antioxidant source to increase rumen ecology of Etawah crossbreed dairy goats. Experiments *in-vitro* using a factorial completely randomized design 3×3 with 4 replications. Factor I consists PrAOx1: 5, PrAOx2: 7.5 and PrAOx3: 10 mg/kg. Factor II consists mineral and vitamin of: $MV_1$: 60 ppm mineral Zn+10 ppm vitamin E, $MV_2$: 15 ppm mineral Cu+10 ppm vitamin E, $MV_3$: 0.15 mineral Se+10 ppm vitamin E. The results showed that there were an interaction between PrAOx and MV on total VFA ($p<0.05$) but not for gas production, pH, $NH_3$, number of bacteria, acetate, propionate and butyrate concentration of Etawah crossbreed dairy goats rumen. The conclusion of this research is the use of an pineapple rind antioxidant by 5 mg/kg plus mineral Zn-vitamin E can be used as a food supplement in Etawah crossbreed dairy goats ration.

**Key words:** Feed supplement, antioxidant, rumen, ecology

## INTRODUCTION

Various industries process agricultural raw materials into processed products that produces biomass such as compost heap or residue that have high economic efficiency potential in ruminants. Industrial processing of pineapple (*Ananas comosus* L. Merr) produce waste in the form of fruit skin that amount quite a lot as a byproduct. Aside consumed as fresh fruit pineapple also widely used as a raw material for various agricultural industries such as jams, candy, syrups and pineapple lunkhead.

Pineapple contains phenolic compounds and β-carotene (Gardner *et al*., 2000; Charoensiri *et al*., 2009). Added by Kongsuwan *et al*. (2009) that pineapple contains vitamin C (6,45-18,88 mg/100 g), β-carotene (1,41-3,35 µg/100 g) and phenols (20,28-26,20 mg GAE/100 g) in total. Pineapple is a nutritious fruit since it contain high amount of vitamin C and natural antioxidants that able to hamper the development of certain clinical conditions including heart disease and cancer in humans (Diplock, 1994; Mahdavi *et al*., 2010). Dried pineapple skin contains antioxidants 38,95 mg/100 g, vitamin C 24, 40 mg/100, β-carotene 59,98 ppm, flavonoids, quercetin 1.48%, 32.69 ppm phenols and 5.29% saponin in total (Mardalena *et al*., 2011).

The animal in optimal conditions will change the balance of microbes in the rumen. therefore it need supplementation of feed containing antioxidants for microbial protein synthesis so that they can get rid of pathogenic microbes. Some antioxidants such as flavonoids, saponins, vitamins and minerals play a role in the metabolic processes that synergistically enhance rumen digestibility of feed in the rumen (Mardalena *et al*., 2013). Wina *et al*. (2005), saponins substances have been reported to have potential to suppress growth of protozoa and change fermentation in the rumen system. Increased digestibility of feed, will increase the content of total VFA and partial (acetic acid, propionic acid and butyric acid), which is a source of energy for the animals.

One of the factors that cause the low productivity of dairy goats in producing milk is feed. Feed in the rumen will undergo fermentation into energy due to the performance of rumen bacteria (Suharti *et al*., 2010) and indirectly cause a decrease in the availability of minerals (Muhtarudin and Liman, 2006). Digestion of food in the rumen occurs by a combination of microbial fermentation and physical breakdown during rumination. Microbial attack is carried out by a mixed population of bacteria and ciliate protozoa, together with a smaller, but possibly metabolically important, population of anaerobic fungi (Dehority, 2004).

The objective of this study is to evaluate of feed supplement contain pineapple rind, micro minerals and vitamin E as antioxidant source to rumen ecology of Etawah crossbreed dairy goats.

## MATERIALS AND METHODS

Rumen fluids were taken from the Etawah crossbreed goat's offspring. Rumen fluid can be taken using a *stomach tube*. *Stomach tube* inserted into the rumen tube through the mouth, with the help of rumen fluid vacuum pumps that can be aspirated and placed on the container bottle. The rumen fluid are to be put in the thermos before taken to the laboratory. Before the rumen fluid were poured inside the thermos, the thermos must

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be filled with hot water first and then the hot water removed right before rumen fluid poured into the thermos.

Medium solution was made to imitate the real conditions in the rumen of the ruminants. This solution used as the media to process the growth and breeding of rumen microbe using in vitro method. The preparation of medium and reduction solution refers to the method of Theodorou and Brooks (1990). The ingredients of the solution are buffer, macro mineral solution, micro mineral solution and resazurin. The Substrate is used and evaluated using *in vitro* methods is pineapple skin which is dried in an oven at 55°C for 72 h and grounded to pass at 1 mm and added a solution of $ZnSO_4$, $CuSO_4$ and vitamin E. Preparation of the solution and microbial counting procedures refers to the method of Ogimoto and Imai (1981). N-$NH_3$ concentration was determined by Conway Microdifusion techniques (General Laboratory Prosedures, 1966). Concentrations of volatile fatty acids (VFA) were measured using gas chromatography (GC Star 3400 CX, Varian and Sugarland, TX, USA). Quantitative pytochemical result can see at Table 1.

Experiments *in-vitro* using a factorial completely randomized design 3×3 with 4 replications. Factor I consists of PR1: pineapple rind antioxidant (PrAOx) 5, PR2: PrAOx 7.5 and PR3: PrAOx 10 mg/kg. Factor II consists of: $MV_1$: 60 ppm mineral Zn+10 ppm vitamin E, $MV_2$: 15 ppm mineral Cu+10 ppm vitamin E, $MV_3$: 0.15 mineral Se+10 ppm vitamin E.

The parameters observed were gas production, pH, $NH_3$ concentrations (mM), number of rumen bacteria (CFU/mL), dry matter digestibility (%), organic matter digestibility (%), total volatile fatty acid and partial concentration (mM). Data were subjected to one-way ANOVA using procedure of SAS (1985). The differences among treatment means ($p<0.05$) was distinguished.

## RESULTS AND DISCUSSION

**Gas production:** Table 1 shows that provision of pineapple rind antioxidant treatment significantly ($p<0.01$) affect gas production. It is shown that the gas production on treatment PrAOx 5 mg/kg is 18:06 mg/kg BW PrAOx higher than treatment PrAOx 7.5 and PrAOx 10 mg/kg.

Gas production is microbe activity parameter in energy and protein synthesis of microbial origin (Prihartini *et al*., 2007). Rumen microbes convert organic acids into VFA accompanied by the formation of gas (Orskov and Ryle, 1990). The gas production rate become higher and gas formation potential is also increasing. The high gas production is an indicator of the formation of VFA which mainly acetic acid and propionic (Menke *et al*., 1979). The gas that has been produced indicate the process of feed fermentation by rumen microbes in which hydrolyze carbohydrate become monosaccharides and disaccharides are then fermented into volatile fatty acids (VFA), especially acetic acid, propionic and butyric and methane ($CH_4$) and $CO_2$ (McDonald *et al*., 2002).

Provision of micro mineral treatment significantly ($p>0.05$) affect gas production but no interaction between treatment administration pineapple rind antioksidan with micro minerals. By giving $MV_1$, the gas production significantly ($p<0.05$) higher than the provision $MV_2$ and $MV_3$. The addition of Zn minerals provide excellent response. This is because the mineral zinc plays a role in the metabolism of nucleic acids and proteins, enzyme replacement process and enzyme activity (Underwood, 1981). The observation results of Durand and Kawashima (1980) on the Zn content of washed suspensions of rumen microbes (Washed suspensions of rumen microorganism = WSRM) is high which indicates that Zn is required by rumen microbes at a high level as well.

Figure 1 shows that the gas production rate represents an increasing of gas production for the entire treatment

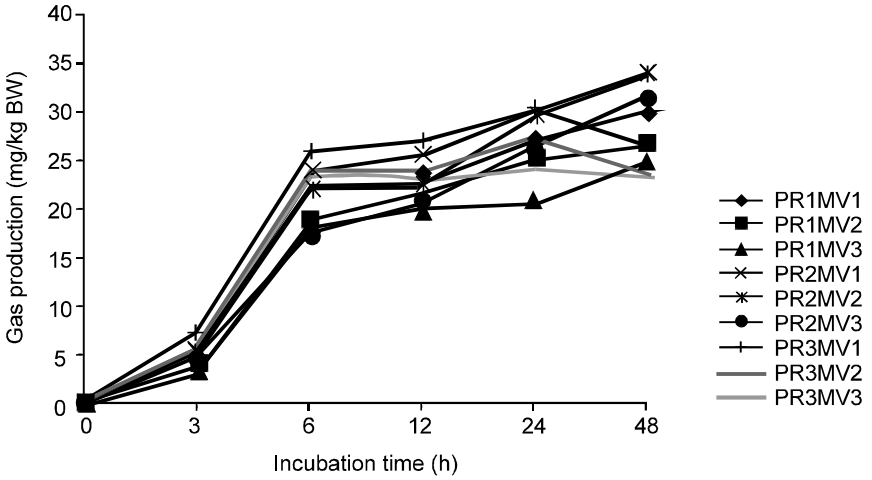


Fig 1: Effect of feed supplement on rumen gas production

Table 1: Effect of feed supplement to gas production, pH, $NH_3$ and bacteria rumen

| Parameters | Pineapple rind | Mineral-Vit E | | | Average | Significancy | | |
|---|---|---|---|---|---|---|---|---|
| | | MV1 | MV2 | MV3 | | PR | MV | PR×MV |
| Gas Production | PR 1 | 18.67 | 18.33 | 17.17 | 18.06 | ** | * | NS |
| (mg/kg BW) | PR 2 | 18.33 | 17.67 | 16.67 | 17.56 | | | |
| | PR 3 | 16.70 | 14.67 | 11.67 | 14.35 | | | |
| SEM = 1.83 | Average | 17.90 | 16.89 | 15.17 | | | | |
| pH | PR 1 | 6.52 | 6.52 | 6.51 | 6.52 | NS | NS | NS |
| | PR 2 | 6.49 | 6.50 | 6.59 | 6.53 | | | |
| | PR 3 | 6.53 | 6.55 | 6.59 | 6.56 | | | |
| SEM = 0.42 | Average | 6.51 | 6.52 | 6.56 | | | | |
| $NH_3$ (mM) | PR 1 | 5.63 | 5.33 | 4.47 | 5.14 | * | NS | NS |
| | PR2 | 5.47 | 5.57 | 5.04 | 5.36 | | | |
| | PR 3 | | 4.81 | 4.83 | 4.84 | | | |
| SEM = 0.58 | Average | 5.32 | 5.24 | 4.78 | | | | |
| Bacteria | PR 1 | 2.45 | 1.85 | 1.18 | 1.83 | * | NS | NS |
| ($x10^9$ CFU/mL) | PR 2 | 1.74 | 1.32 | 1.23 | 1.43 | | | |
| | PR3 | 1.12 | 0.85 | 0.84 | 0.94 | | | |
| SEM = 0.60 | Average | 1.77 | 1.34 | 1.08 | | | | |

(Source of : Mardalena *et al.*, 2013)
NS = not significant, SEM = standard error mean, * = ($p<0.05$%), ** = ($p<0.01$), PR = pineapple rind, MV = mineral-vit E.
PR1: pineapple rind antioxidant (PrAOx) 5 mg/kg
PR2: pineapple rind antioxidant (PrAOx) 7.5 mg/kg
PR3 : pineapple rind antioxidant (PrAOx) 10 mg/kg
MV1: mineral Zn-vitamin E (Zn-E) as much as 60 ppm-10 ppm
MV2: Mineral Cu-vitamin E (Cu-E) as much as 15-10 ppm
MV3: Minerals Se-vitamin E (Se-E) as much as 0.15-10 ppm

Table 2: Effect of feed supplement to VFA, acetate, propionate and butyrate concentration

| Parameters | Pineapple rind | Mineral | | | Average | Significancy | | |
|---|---|---|---|---|---|---|---|---|
| | | MV1 | MV2 | MV3 | | PR | MV | PR x MV |
| Total VFA (mM) | PR1 | 54.45 | 54.14 | 51.68 | 53.42 | * | ** | * |
| | PR2 | 53.85 | 53.55 | 52.22 | 52.21 | | | |
| | PR3 | 54.42 | 53.78 | 52.18 | 53.46 | | | |
| SEM = 0.79 | Average | 54.24 | 53.82 | 52.03 | | | | |
| Asetate (mM) | PR1 | 27.1 | 25.8 | 24.3 | 25.73 | * | * | NS |
| | PR2 | 26.8 | 26.3 | 25.6 | 26.23 | | | |
| | PR3 | 26.7 | 16.4 | 15.8 | 19.63 | | | |
| SEM = 0.77 | Average | 26.87 | 22.33 | 22.40 | | | | |
| Propionate (mM) | PR1 | 17.4 | 16.5 | 16.5 | 16.80 | ** | ** | NS |
| | PR2 | 16.5 | 16.3 | 15.1 | 15.97 | | | |
| | PR3 | 15.6 | 15.2 | 14.9 | 15.23 | | | |
| SEM = 0.69 | Average | 16.50 | 16.0 | 15.50 | | | | |
| Butyrate (mM) | PR1 | 10.2 | 10.3 | 8.2 | 9.57 | * | * | NS |
| | PR2 | 10.6 | 9.9 | 9.3 | 9.93 | | | |
| | PR3 | 10.3 | 9.4 | 9.3 | 9.67 | | | |
| SEM = 0.83 | Average | 10.37 | 9.83 | 8.97 | | | | |

(Source of : Mardalena *et al.*, 2013)
NS = not significant, SEM = standard error mean, * = ($p<0.05$%), ** = ($p<0.01$), PR = pineapple rind, MV = mineral-vit E.
PR1: pineapple rind antioxidant (PrAOx) 5 mg/kg
PR2: pineapple rind antioxidant (PrAOx) 7.5 mg/kg
PR3: pineapple rind antioxidant (PrAOx) 10 mg/kg
MV1 : mineral Zn-vitamin E (Zn-E) as much as 60-10 ppm,
MV2: Mineral Cu-vitamin E (Cu-E) as much as 15-10 ppm
MV3: Minerals Se-vitamin E (Se-E) as much as 0.15-10 ppm

occurred at incubation time 0-24h and the incubation time 24-48 hours in the treatment $PR_1$ $MV_1$ that shows an increase in the rate of gas production meanwhile in the treatment $PR_3$ $MV_3$ gas production rate decreased.

**pH, $NH_3$ and number of bacteria:** The results shows that giving antioxidant from pineapple rind significantly ($p<0.05$) affect $NH_3$ and the amount of rumen bacteria not significantly ($p>0.05$) affect rumen pH. Provision of micro mineral treatment did not significantly ($p>0.05$),

affects the pH, $NH_3$ and the number of bacteria in the rumen. Interaction between treatment administration pineapple rind antioxidants and micro minerals not significant ($p>0.05$), affecting gas production, pH, $NH_3$ and the number of bacteria in the rumen. $NH_3$ and the number of bacteria consecutively visible in treatment PrAOx 5 mg/kg and 1.77 NM as 5:32×109 CFU/mL higher than treatment of PrAOx 7.5 mg/kg and PrAOx 10 mg/kg. Pineapple rind antioxidant in form of flavonoids and saponins able to increase the number of bacteria. The high concentration of $NH_3$ at $PR_1$ treatment illustrates the high activity of bacteria in the rumen and illustrates that the protein has a high solubility feed that it can be easily degraded by rumen microbes. The optimum concentration of $NH_3$ in the rumen between 85-300 mg/L or 6-21 mM (McDonald *et al*., 2002). It indicates that the concentrations of $NH_3$ generated in this study are still below the ideal range and affect the metabolic activity in the rumen.

**Total and partial VFA:** The various results show that VFA concentration are significantly ($p<0.05$) affected by the pineapple rind antioxidant treatment and very significantly ($p<0.01$) affected by micro mineral. The interaction from these two treatments significantly ($p>0.05$) affect the total VFA. The same thing also happen to propionate consentration that it is very significantly ($p<0.01$) affected by the pineapple rind and micro mineral treatment, but the interaction between those two treatment didn't affect propionate consentration. Concentrations of acetate and butyrate significantly ($p<0.05$), affected by the pineapple rind antioxidant treatment and micro minerals but does not affect the interaction of the two treatments acetate and butyrate concentrations. Table 2 shows that treatment PrAOx 5 mg/kg for each variable is 53.42 mM VFA, 16.80 mM propionate, acetate and butyrate 25.73 mM 7.57 mM in total markedly higher than the treatment of PrAOx 7.5 mg/kg and PrAOx 10 mg/kg. Substrates are available in a pineapple rind is a component of cellulose and hemicellulose fibers. These conditions indicate the existence of an increase in the microbial population. According to Clarke *et al*., 1977, selulotik bacterial cellulose and hemicellulose requires substrates to produce energy for ruminants in the form of VFA. The final process of fermentation is Volatile Fatty Acid, they are acetic acid and propionic acid and used for energy and carbon sources. Acetic acid is a milk fat precursor, body fat and energy (McNamee, 1996). Propionic acid is one's factor for glucose synthesis. Propionic acid concentration will increase if they had consumption a lot of starch and sugar.

Administration of Zn mineral-vitamin E produced a total of 54.24 mM VFA, propionate 16.50 mM, 26.87 mM acetate and 10.37 mM butyrate significantly ($p<0.05$) higher than Cu minerals-vitamin E and Se mineral-vitamin E. Supriyati *et al*. (2000) stated that the addition of 5 ppm Zn micro minerals provides the best response to the rumen microbial activity. In general, the pH value decreases for the addition of mineral treatment, whereas decreased levels of ammonia and total VFA production increased with the addition of a combination of elements Zn, Cu and Mo. Zn supplementation may increase the bacterial population in the rumen due to the needs of the bacteria will be very high Zn is 100-120 mg/kg, so that it able optimize the bacteria in producing digestive enzymes.

**Conclusion:** The level of 5 mg/kg BW pineapple rind antioxidant and 60 ppm mineral Zn+10 ppm Vitamin E improve to good condition of rumen ecology. It showed to increase bacteria rumen, $NH_3$ concentration, total VFA, acetic acid and propionic acid. Condition of pH rumen is showed in normal condition.

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